- c) tagging the analyte capture complex with a fluorescent label;
- d) illuminating the microscopic sorbent zone with a laser in the absence of liquid; and
- e) detecting fluorescence emissions from any microscopic sorbent zone having an analyte capture complex tagged with a fluorescent label, thereby determining the analyte mass harvested from the defined volume of sample.
- 23. (Twice Amended) An analyte binding array for harvesting analyte from a liquid sample, the array comprising a plurality of microscopic sorbent zones immobilized on a substrate, wherein a microscopic sorbent zone comprises a multilayer matrix of an analyte binding partner, the analyte binding partner being present in an amount sufficient to substantially deplete the analyte from a sample and concentrate the analyte on the microscopic sorbent zone, the microscopic zone being from about 60 to about 500 μm in diameter and the sample containing about 10<sup>10</sup> to about 10<sup>10</sup> molecules of analyte per 100 μl of the sample, wherein a volume of the sample is from 20 to 500 μl.
- 26. (Twice Amended) A kit for use in a binding assay that senses analyte mass in a liquid sample of a defined volume, comprising an analyte binding array and a container comprising labeled binding partner,

wherein the analyte binding array comprises a plurality of microscopic sorbent zone sorbent zones immobilized on a substrate, wherein a microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, the analyte binding partner being present in excess relative to the analyte, so that any analyte present in the defined volume of the sample is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner, and

the labeled binding partner having a fluorescent label and being capable of binding to an analyte bound by an analyte binding partner.





## Please add the following new claims 29-36:

- 29. (New) The assay of claim 4, wherein the immobilizing step a) further comprises:
  - al) derivatizing the binding partner with a photolabile linker moiety to obtain a derivatized binding partner;
    - a2) drying the derivatized binding partner on the substrate; and
    - a3) exposing the substrate to UV radiation.
- 30. (New) The assay of claim 14, wherein the amount of the analyte binding partner immobilized in the sorbent zone with a diameter from 60  $\mu$ m to 500 $\mu$ m is from 10<sup>9</sup> to 10<sup>12</sup> molecules.
- 31. (New) The analyte binding array of claim 23, wherein the amount of the analyte binding partner immobilized in the sorbent zone is from 10<sup>9</sup> to 10<sup>12</sup> molecules.
- 32. (New) The kit of claim 26, wherein the amount of the analyte binding partner immobilized in the sorbent zone with a diameter from 60  $\mu$ m to 500 $\mu$ m is from 109 to 1012 molecules.
- 33. (New) A binding assay for sensing analyte mass in a liquid sample, comprising:
- a) immobilizing an array on a substrate, wherein the array comprises a plurality of microscopic sorbent zones, wherein each microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, wherein the amount of the analyte binding partner immobilized in the sorbent zone with a diameter from 60 µm to 500 µm is from 109 to 1012 molecules;
- b) contacting a defined volume of sample believed to contain an analyte with at least one microscopic sorbent zone, whereby analyte present in the defined volume is substantially depleted from the sample and concentrated on the

microscopic sorbent zone to form an analyte capture complex with the analyte binding partner;

- c) tagging the analyte capture complex with a fluorescent label; and
- d) detecting fluorescence emissions from the microscopic sorbent zone to determine the analyte mass harvested from the defined volume of sample.
- 34. (New) A binding assay for sensing analyte mass in a liquid sample, comprising:
  - a) derivatizing a binding partner with a photolabile linker moiety to obtain a derivatized binding partner;
  - b) applying aliquots of the derivatized binding partner to a substrate;
- c) exposing the substrate to UV radiation to immobilize the analyte binding partner, whereby an array of microscopic sorbent zones comprising the analyte binding partner forms;
- d) contacting a defined volume of sample believed to contain an analyte with at least one microscopic sorbent zone, the analyte binding partner in the microscopic sorbent zone being present in excess relative to the analyte, so that any analyte present in the defined volume is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner;
  - e) tagging the analyte capture complex with a fluorescent label;
- f) illuminating the microscopic sorbent zone with a laser in the absence of liquid; and
- g) detecting fluorescence emissions from any microscopic sorbent zone having an analyte capture complex tagged with a fluorescent label, thereby determining the analyte mass harvested from the defined volume of sample.
- 35. (New) An analyte binding array for harvesting analyte from a liquid sample, the array comprising a plurality of microscopic sorbent zones immobilized on a substrate, wherein a microscopic sorbent zone comprises an analyte binding

